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(54) **Method, device and mixture for aerial application of Trichogramma parasitoids in plant protection, and evaluation method of aerial application of Trichogramma parasitoids**

(57) A method of the aerial application of parasitoids of the genus *Trichogramma* in plant protection, in which on a defined plant surface area or on a unit of the plant surface area a defined amount of *Trichogramma* parasitoids is distributed by a flying means. First of all, a loose mixture consisting of the pupae of the parasitoids *Trichogramma*, which are in defined developmental stages, and of loose inert substrate of natural origin is formed, whereupon the loose mixture is gradually released in a defined amount per unit of time from the aerial means flying at a defined speed and at a defined altitude over the plant surface treated.

An evaluation method of the aerial application of *Trichogramma* parasitoids in plant protection, in which on a defined area of vegetation or on a unit of the area of vegetation a defined amount of *Trichogramma* parasitoids is distributed by means of a flying means. Before the application of the loose mixture consisting of the pupae of the parasitoid *Trichogramma*, which are in different defined developmental stages, and of a loose inert substrate of natural origin, a set of small capturing pads (4) of the loose mixture is distributed on a defined area of the plant surface treated, whereupon the application of *Trichogramma* parasitoids is performed with the aid of the flying means and subsequently the number of the pupae of *Trichogramma* parasitoids caught on individual capturing pads (4) is optically evaluated.

A device for the aerial application of *Trichogramma* parasitoids in plant protection, which comprises a reservoir of *Trichogramma* parasitoids and a dispenser. The reservoir of *Trichogramma* parasitoids is composed of a gravity reservoir of the loose mixture of the pupae of the parasitoid *Trichogramma*, which are in defined developmental stages, and of a loose inert substrate of natural

origin. Arranged at the outlet of the gravity reservoir of the loose mixture is a dispensing mechanism (3) of the loose mixture, whereby the device (1) is provided with means for fastening to the flying means.

A loose mixture for the aerial application of *Trichogramma* parasitoids in plant protection. The loose mixture consists of the pupae of the *Trichogramma* parasitoid, which are in defined developmental stages, and of a loose inert substrate of natural origin.

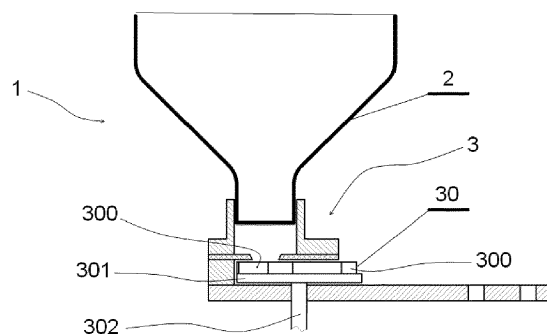


Fig. 2a

Description

Technical field

[0001] The invention relates to a method of aerial application of parasitoids of the genus *Trichogramma* in plant protection, in which over a defined plant surface area or over a unit of plant surface area a defined amount of *Trichogramma* parasitoids is distributed by a flying means.

[0002] The invention also relates to a method of evaluation of aerial application of *Trichogramma* parasitoids in plant protection, in which over a defined plant surface area or over a unit of plant surface area a defined amount of *Trichogramma* parasitoids is distributed by a flying means.

[0003] The invention further relates to a device for aerial application of *Trichogramma* parasitoids in plant protection, which contains a reservoir of *Trichogramma* parasitoids and a dispenser.

[0004] The invention also relates to a loose mixture for aerial application of *Trichogramma* parasitoids in plant protection.

Background art

[0005] *Trichogramma* egg parasitoids are specialized in parasitization of eggs of hosting species of insects, particularly butterflies. Due to this ability they have been used in the sphere of biological plant protection for several decades, i.e. they have been acting as biological control agents against pests, namely against caterpillars of butterflies. Nowadays, *Trichogramma* parasitoids are most widely applied in the sphere of maize protection from the European corn borer. *Trichogramma* parasitoids are used to control this pest in such a manner that a defined amount of the pupae of the parasitoid per unit of the maize field is distributed over the maize field during the initial phase of laying eggs by a particular pest, i.e. in this case for example by females of the European corn borer. After females of the parasitoid hatch from the pupae of the parasitoid, they actively, by smelling within a limited action area (tens of centimetres up to individual metres), search for clusters of eggs laid by the pest. The females of the parasitoid lay their own eggs into the eggs of the pest, whereupon inside this parasitized egg of the pest (the host) a parasitoid of the genus *Trichogramma* completely develops in a relatively short period of time - in approximately 10 days, whereby, simultaneously, the embryo of the pest is destroyed, i.e. the embryo of the caterpillar of the European corn borer, by which means the occurrence of the European corn borer in the maize field treated is eliminated, and, consequently, the maize field is in this manner protected from this pest and the plant surface area thus protected is not damaged.

[0006] As *Trichogramma* parasitoids due to the extreme miniaturization of their body size hatch only with minimum energy reserves (fats, sugar) and move mainly

by walking short distances (tens of centimetres up to individual metres), it is necessary to distribute a sufficient amount of *Trichogramma* parasitoids per unit of the plant surface area treated in order to ensure that each plant of the field treated is subjected to the acting of *Trichogramma* parasitoids, which have a very limited action radius, since *Trichogramma* parasitoids have to have enough energy for their existence to be able to search for the clusters of the pest's eggs (hosting eggs) on the plants and to be able to parasitize them.

[0007] Nowadays, there are several methods of the application of *Trichogramma* parasitoids. The first known method of the application of *Trichogramma* parasitoids is dropping or throwing the pupae of the parasitoids into the axil of the maize leaves by hand, whereby the pupae of the parasitoid in the form of "powder" have a particle size of approximately 0.7 mm. A second known method of the application of *Trichogramma* parasitoids is the placing of a defined amount of *Trichogramma* parasitoids on paper cards or into capsules of various constructions, which are subsequently distributed again by hand in the maize field in accordance with the requirements per unit of the area of the plot protected.

[0008] However, these methods have a number of disadvantages which can be roughly divided into two categories: organizational and technical problems and biological disadvantages.

[0009] The organizational and technical problems are connected with financial and organizational demands of plant protection, as it is highly labour intensive, when 1.5 man hour/ha is needed for the protection of the area. Owing to the fact that the biological plant protection by means of *Trichogramma* parasitoids requires relatively precise timing of the application of the parasitoids, and, what is more, due to the fact that the suitable time for the application is approximately 2 - 3 days from the point of view of the time point of the beginning of laying the eggs by the particular pest, it is practically impossible to treat large plant surface areas, such as maize fields, sometimes covering areas of up to thousands of hectares. In the case of such vast plant surface areas protected, using *Trichogramma* parasitoids for biological protection would require employing an enormous quantity of labourers in an extremely short period of time, which brings not only enormous workforce costs, but also involves costs to cover the workforce activation, accommodation and other living conditions.

[0010] The biological disadvantages include the occurrence of unprotected clusters of a relatively large amount (approximately 1.000 - 1.500 p. in one place) of the pupae of the parasitoids of *Trichogramma* parasitoids in one place of the field protected using the existing described methods of application of *Trichogramma* parasitoids on the plant surface area, since *Trichogramma* parasitoids are highly attractive for predaceous insects (lady birds, predaceous bugs), which causes considerable losses in the order of as many as tens of percent during the time period from the application of *Trichogramma* parasitoids

to the hatching of *Trichogramma* parasitoids from the pupae, which both increases the costs of biological plant protection by means of *Trichogramma* parasitoids, and decreases the efficiency of biological plant protection by means of *Trichogramma* parasitoids. Although this biological drawback is reduced by the encapsulation of the pupae of *Trichogramma* parasitoids into capsules, this does not solve the existence of another major shortcoming, namely the demanding and slow distribution of *Trichogramma* parasitoids on the plant surface, e.g. in maize fields.

[0011] There are also known methods, devices and means for aerial application of a parasitoid of the genus *Trichogramma*, for example according to US patents No 978 615, 3 872 012, 3 968 933, 3 994 437, 4 089 441, 4 260 108, 4 475 819, 4 487 365, 4 966 329, 5 148 989, 5 190 225, 5 213 271, 5 288 028, 5 794 847, 4 537 333 and others.

[0012] In principle, they describe different solutions to the problem of releasing biologically active insects in required growth stages, for example *Trichogramma* parasitoids, and the maximum possible uniform distribution on the area treated. For this purpose, a variety of applicators is used, which comprise a dispenser connected to a "spraying" device, into which during the flight of the aircraft the surrounding air gets spontaneously, which evokes a stir-up of the individuals of biologically active insects in the air in the spraying device and by the subsequent releasing of this mixture of the air and the biological material from the spraying device the biological material is distributed on the area treated. These solutions are demanding in terms of design and construction, as well as operation.

[0013] According to one of the above-mentioned patents, the biologically active insects are treated with a layer of adhesive material so that the sprayed biological material is better captured on the plants, by which means the method is supposed to be more efficient. However, this makes the whole method more expensive and, in addition, the practical effect of the adhesive material on the individuals of the sprayed insects after hatching from the pupae is disputable.

[0014] This document also describes a method of evaluation of the quality of distribution of the applied biologically active insects by means of a long stripe of capturing material divided by marks into sections one meter long located on plywood units and cardboard, which are carried to the place of the insect application in plastic vessels and are distributed together with leaves of walnuts into the monitored area of the insect application. After that an aircraft with a set dispenser of biologically active insects flies over the given area and by means of a hand lens (7 times enlargement) the number of captured individuals of the sprayed insects is evaluated. Simultaneously, the capturing material and the walnut leaves are taken to a laboratory for 7-day incubation. Afterwards another detailed analysis of the presence of the eggs of the applied insects - the parasites - is carried out, and according to

the number of eggs with holes the percentage of successful hatching of an individual of the applied insects per unit area of the capturing material is determined, which is essential for the overall assessment of the efficiency of the application of biologically active insects in the field treated. This method is extremely complicated, time-consuming and requires special equipment and is not able to provide relevant data about the quality of the application immediately and on the spot of the application.

[0015] The aim of the invention is to eliminate or at least minimize the disadvantages of the background art in the sphere of aerial application of *Trichogramma* parasitoids, and, above all, to enable fast, functional, efficient and checkable distribution of *Trichogramma* parasitoids in on the plant surface area protected in the required amount and at the required time, all this being performed under acceptable economic conditions.

Principle of the invention

[0016] The goal of the invention is achieved by a method of the application of *Trichogramma* parasitoids in plant protection, whose principle consists in that at first a loose mixture consisting of the pupae of *Trichogramma* parasitoids, which are in defined developmental stages, and of a loose inert substrate of natural origin having a required concentration is created, whereupon the loose mixture is released gradually in defined amounts per unit of time from a flying means at a defined speed and at a defined altitude over the plant surface area treated.

[0017] The principle of the evaluation of the application of *Trichogramma* parasitoids in plant protection consists in that before the application of the loose mixture consisting of the pupae of the parasitoid of the genus *Trichogramma*, which are in defined developmental stages, and a loose inert substrate of natural origin, a system of small capturing pads of the loose mixture is distributed on a defined area of the plant surface treated, whereupon the application of *Trichogramma* parasitoids by means of a flying means is carried out and subsequently the number of the pupae of *Trichogramma* parasitoids captured on individual capturing pads is optically evaluated. The principle of the device for the application of *Trichogramma* parasitoids in plant protection consists in that the reservoir of *Trichogramma* parasitoids is composed of a gravity reservoir of the loose mixture consisting of the pupae of parasitoids *Trichogramma*, which are in defined developmental stages, and of a loose inert substrate of natural origin, at whose outlet a dispensing mechanism of the loose mixture is arranged, whereby the mechanism is equipped with means of fastening on the flying means.

[0018] The principal of the mixture for application of *Trichogramma* parasitoids in plant protection consists in that it consists of a loose mixture of the pupae of the parasitoids *Trichogramma*, which are in defined developmental stages, and of a loose inert substrate of natural

origin.

[0019] The advantage of this invention is a possibility to treat even vast areas of vegetation quickly, reliably and with a defined precision, whereby it is possible to achieve high efficiency of biological protection of plants at relatively low costs.

Description of drawings

[0020] The invention is schematically represented in the drawing, where Fig. 1 shows the structure of the loose mixture, Fig. 2a is a side view of a schematic arrangement of the dispensing mechanism of the loose mixture, Fig. 2b is a top view of a schematic arrangement of the dispensing mechanism of the loose mixture without a gravity reservoir of the loose mixture and Fig. 3 shows the distribution of the control capturing pads for the evaluation of the application.

Specific description

[0021] The invention will be described on an example of the application of *Trichogramma parasitoides* in a loose mixture with a loose inert substrate of natural origin, where the application is carried out by means of a device for the application of the loose mixture for plant protection, whereby the device for the application of the loose mixture is attached to a flying means, e.g. an airplane, and the quality and/or homogeneity of the application is evaluated by a method according to the present invention.

[0022] The device for the application of *Trichogramma parasitoids* in this loose mixture in plant protection (see Fig. 2) comprises a dispenser **1** of loose materials with a gravity reservoir **2** of the loose mixture.

[0023] The loose mixture includes a loose inert substrate of natural origin and the pupae of the parasitoid *Trichogramma* in precisely defined developmental stages, which determine the dynamics of hatching of the parasitoid in time. The loose inert substrate of natural origin includes, for example, barley corn or a mixture of barley corn and other grain products or some other material of natural origin, whereby the reason why the loose inert substrate of natural origin according to this invention is used is the fact that it has no negative influence on the pupae of the parasitoid *Trichogramma*, nor has it any negative impact on the environment in the area of the aerial application of the loose mixture of the pupae of the parasitoid *Trichogramma* and the loose inert substrate of natural origin. Another reason for employing the loose inert substrate of natural origin according to the present invention is the fact that it ensures the required physical parameters of the resulting loose mixture, especially its looseness etc. In order to achieve and maintain the physical properties of the resulting loose mixture it is advantageous to use a loose inert substrate having equal size grains or grains whose size is similar to that of the pupae of the parasitoid *Trichogramma*.

[0024] When preparing the loose mixture according to the invention a required rate of individual components of the loose mixture is set, i.e. the rate of the pupae of the parasitoid *Trichogramma* and the loose inert substrate of natural origin according to the required amount of the individuals of the parasitoid *Trichogramma* on a unit of area of the future protected plot of vegetation, for example 120.000 - 150.000 individuals/ha, which leads to the formation of a mixture of the pupae of the parasitoid and the loose inert substrate having a required concentration of the pupae of the parasitoid in a unit of capacity of the resulting loose mixture, which is shown in Fig. 1.

[0025] The resulting loose mixture of the pupae of the parasitoid *Trichogramma* and of the loose inert substrate of natural origin is inserted into the reservoir **2** of the loose mixture of the dispenser **1**. The dispenser **1** is adjustable in relation to the amount of the loose mixture given out per unit of time and is located on the flying means, such as an airplane. According to the concentration of the parasitoid of the genus *Trichogramma* in the resulting loose mixture and according to the required quality and/or uniformity of the application in accordance with the current meteorological conditions, such as the power and direction of the wind, humidity, etc., the required speed is determined, the altitude and direction of the flight of the flying means with an installed dispenser **1**, which is equipped with means of fastening to a suitable part of the aerial means, such as an airplane, a helicopter, remotely controlled airplane, an automatic aerial means, etc., for example to a landing gear or a fuselage, or under a wing or wings, to a tail, or to another suitable part of the flying means etc. One flying means may comprise one dispenser **1** or more dispensers **1**, e.g. two or three, etc., attached to it.

[0026] The drawing shows a dispenser **1** of the resulting loose mixture of the pupae of the parasitoid *Trichogramma* and the loose inert substrate of natural origin, which is provided with a gravity dispenser **2** of the loose mixture of the pupae of the parasitoid *Trichogramma* and the loose inert substrate of natural origin. Assigned to the lower end of the gravity reservoir **2** is a dispensing mechanism **3** of the loose mixture with a rotating dosing disc **30** for measuring individual incremental doses of the loose mixture of the pupae of the parasitoid *Trichogramma* and of the loose inert substrate of natural origin. The dosing disc **30** is loosely slipped over a carrier **301**, which is mounted on an output shaft **302** of an unillustrated engine. On the circumference of the dosing disc **30** there are five dents **300** for measuring the delivered dose of the loose mixture, and so during the rotation of the dosing disc **30** the dents **300** are gradually filled with material from the gravity reservoir **2** of the loose mixture. During the further rotation of the dosing disc **30** the measured loose mixture is freely discharged from the dents **300** to the air and falls down onto the treated area of the crops. Apart from the regulation of the speed and altitude of the flight, the speed and amount of the released loose mixture can be regulated by the speed of releases and the

amount of the released mixture, which can be performed in the illustrated embodiment of the dispenser **1** by setting the revolutions of the rotating dosing disc **30** and/or by changing the size of the dents **300** of the dosing disc **30**, for example by replacing one dosing disc **30** with a certain number of dents **300** of a certain size with another perforated disc **30** with a different number of and/or a different size of the dents **300**.

[0027] The homogeneity and quality of the application of the required amount of the pupae of *Trichogramma* parasitoids per unit area of the plant surface treated can be verified by a method according to the invention, in which on a free area, such as the area of an airport, etc., or directly on the protected plant surface, evenly in a defined mutual distance, e.g. 1 m, perpendicularly to the direction of the flight of the flying means with a determined number of dispensers **1**, capturing pads **4** of the loose mixture are distributed, e.g. it is possible to use A5 size plastic plates provided with a sticky coating on their upper surfaces, whereby the capturing pads **4** can be preferably any colour except black, since the evaluated pupae of *Trichogramma* parasitoids are dark or black, and the colour of the loose inert substrate of natural origin is usually light (including the range of white to yellow and light brown).

[0028] In order to increase the information value of the evaluation of the homogeneity and quality of the application the capturing pads **4** are distributed in a geometrically suitable arrangement in relation to the direction of the flight **S** of the flying means, e.g. in a matrix arrangement, or in the form of a letter of the alphabet, especially the form of the letter "Z" is advantageous, etc. These capturing pads **4** are evaluated after the flight of the flying means by the naked eye or with the aid of a magnifying glass regarding the number of the pupae of *Trichogramma* parasitoids caught on individual capturing pads **4**. In this manner it is possible to verify the quality and homogeneity of just performed application immediately on the spot after the application of the pupae of *Trichogramma* parasitoids, i.e. to verify the amount of the pupae of *Trichogramma* parasitoids per unit area and also check the homogeneity of the performed application and, if needed, e.g. in case the required homogeneity and amount have not been achieved due to an abrupt climatic change (a sudden gust of wind) it is possible to carry out without delay a repeated application with the same or modified parameters of the application, such as the speed and altitude of the flight, the amount of the released loose mixture per unit of time etc. It is apparent that the flying means flies over the treated area there and back in gradually shifting stripes, by which means the application is performed on the entire area of the plant surface treated.

[0029] By setting the concentration of the loose mixture appropriately, as well as by setting the amount of the loose mixture dosed by the dispenser **1** per unit of time, the speed and altitude of the flight of the particular type of aircraft used, etc., it is possible to achieve the application of a verified and guaranteed number of the pupae

of *Trichogramma* parasitoids per unit area of the plant surface treated, e.g. 100.000 - 250.000 of individuals/ha. In order to achieve a more precise navigation of the flight of the flying means, especially in order to prevent the occurrence of untreated spots due to not observing the maximum allowed distance between individual flights of the flying means over the plant surface area treated, it is advantageous to use a suitable positioning system, for example the system GPS, GLONASS, GALILEO etc., which brings another benefit, namely the precise control of the flight with the possibility of its automation, and also the possibility of recording the flight path, including individual transits in individual stripes over the plant surface treated, which, for example, enables feedback control of the flight path, etc.

[0030] It follows from the applicant's experience that the optimum application of the pupae of *Trichogramma* parasitoids constitutes 1 pupa of the *Trichogramma* parasitoid per approximately every 7 dm² of the area of the plant surface treated. For example, when seeding maize with the spacing 60 x 10 cm the average distance of the hatched individual of a *Trichogramma* parasitoid to the most distant plant of maize, which each individual of a *Trichogramma* parasitoid is to "search", is in the order of decimetres, which is an optimum state, leading to the 75 - 95 % efficiency of protection according to this invention. This density of distribution of the pupae of *Trichogramma* parasitoids considerably reduces the risk of the individuals of *Trichogramma* parasitoids being found and destroyed by predators, such as ladybird beetles, predaceous bugs, ants, etc.

[0031] As an experiment, the applicant achieved the treatment of a field having an area of approximately 4.3 ha/minute, the price of the application being approximately 140 crowns/ha, the real application capacity being approximately 1.000 ha/ 8 hrs., using one agricultural aircraft. These values are experimental values, achieved with the illustrated embodiment of the dispenser in Fig. 2, with a particular concentration of the loose mixture (18 % *trichogramma* and 82 % semolina) and with the aid of

a particular type of aircraft (Z 37A Čmelák). It is obvious that by the appropriate setting of these parameters, it is possible to influence the areal application capacity.

[0032] For example, to meet the requirement of applying 120,000 - 150,000 individuals of the *Trichogramma* parasitoid on a treated area of 1 ha, the selected type of the loose mixture will have the concentration of 18 % *Trichogramma* and 82 % semolina, and at the selected speed of the flying means or at the speed which is known beforehand according to the type of the flying means and depending on the selected altitude of the flight of 4 - 6 m over the ground, the set amount of the released loose mixture per 1 minute will be 100 ml, and so a dosing disc **30** will be used, which is 8 mm thick and whose diameter is 48 mm and which has 5 dents **300** with a diameter of 20 mm. Under these conditions the application of the required amount of individuals of the *Trichogramma* par-

asitoid on the treated area is achieved. It is apparent that the varying factors include especially the concentration of the loose mixture, the speed of the flight of the flying means and the speed and amount of the released loose mixture. If needed, it is possible to use a flying means with two or more dispensers 1.

Claims

1. A method of aerial application of parasitoids of the genus *Trichogramma* in plant protection, in which a defined amount of *Trichogramma* parasitoids is distributed on a defined plant surface area or a unit of plant surface with the aid of a flying means, **characterized in that** at first, a loose mixture consisting of the pupae of the parasitoid *Trichogramma*, which are in defined developmental stages, and of a loose inert substrate of natural origin with a required concentration is formed, whereupon the loose mixture is gradually released in a defined amount per unit time from the aerial means flying at a defined speed and at a defined altitude over the plant surface treated. 5
2. A method according to Claim 1, **characterized in that** the loose mixture is released in incremental doses of a defined size. 10
3. A method according to Claim 1 or 2, **characterized in that** the loose mixture is released from one or more dispensers (1) attached to the flying means. 15
4. A method according to Claim 1, **characterized in that** the movement of the flying means is monitored and/or controlled by a positional device with a data record. 20
5. A method of evaluation of aerial application of *Trichogramma* parasitoids in plant protection, in which a defined amount of *Trichogramma* parasitoids is distributed on a defined plant surface area or a unit of plant surface with the aid of a flying means, **characterized in that** before the application of the loose mixture consisting of the pupae of the parasitoid *Trichogramma*, which are in defined developmental stages, and of a loose inert substrate of natural origin a system of small capturing pads (4) of the loose mixture is distributed on a defined area of the plant surface, whereupon the application of *Trichogramma* parasitoids with the aid of the flying means is carried out and subsequently the number of the pupae of *Trichogramma* parasitoids caught on individual capturing pads (4) is optically evaluated. 25
6. A method according to Claim 5, **characterized in that** capturing pads (4) are formed by a5 size plastic plates provided with a sticky coating on their upper surfaces. 30
7. A method according to Claim 5, **characterized in that** the system of small capturing pads (4) has matrix arrangement or is arranged in the form of a letter of the alphabet. 35
8. A device for aerial application of *Trichogramma* parasitoids in plant protection, which comprises a reservoir of *Trichogramma* parasitoids and a dispenser **characterized in that** the reservoir of *Trichogramma* parasitoids is composed of a gravity reservoir of the loose mixture of the pupae of the parasitoid *Trichogramma*, which are in defined developmental stages, and of a loose inert substrate of natural origin. Arranged at the outlet of the gravity reservoir is a dispensing mechanism (3) of the loose mixture, whereby the device (1) is provided with means for fastening to the flying means. 40
9. A device according to Claim 8, **characterized in that** the dispensing mechanism (3) of the loose mixture is provided with a rotating perforated disc (30) or with a replaceable rotating perforated disc (30) with through cylindrical holes (300) for measuring the released doses of the loose mixture, whereby the perforated disc (300) has adjustable revolutions. 45
10. A device according to Claim 8, **characterized in that** the flying means is provided with a device for recording data from the positional system. 50
11. A loose mixture for the aerial application of *Trichogramma* parasitoids in plant protection, **characterized in that** it is composed of the pupae of the *Trichogramma* parasitoid, which are in defined developmental stages, and of a loose inert substrate of natural origin. 55
12. A loose mixture according to Claim 11, **characterized in that** the loose inert substrate of natural origin is formed by cereals.
13. A loose mixture according to Claim 12, **characterized in that** the loose inert substrate of natural origin is formed by semolina.

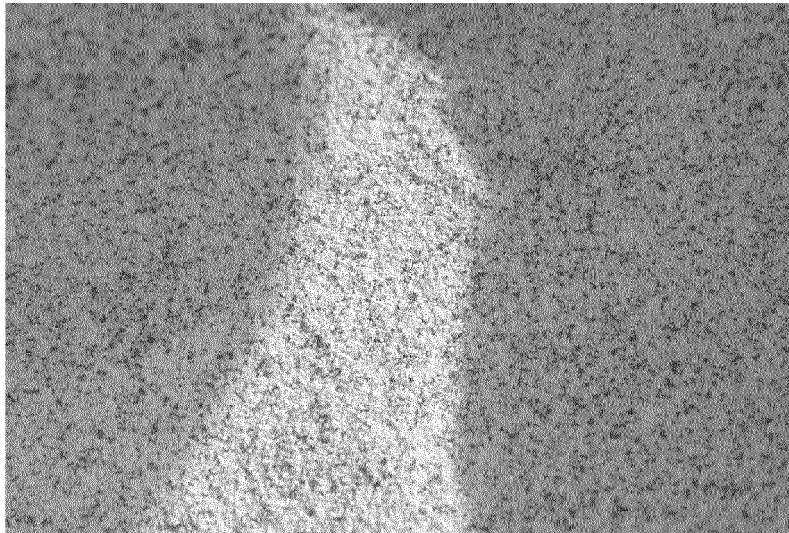


Fig. 1

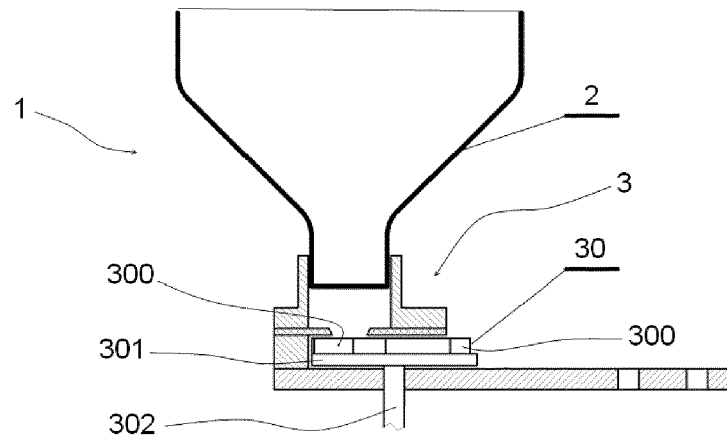


Fig. 2a

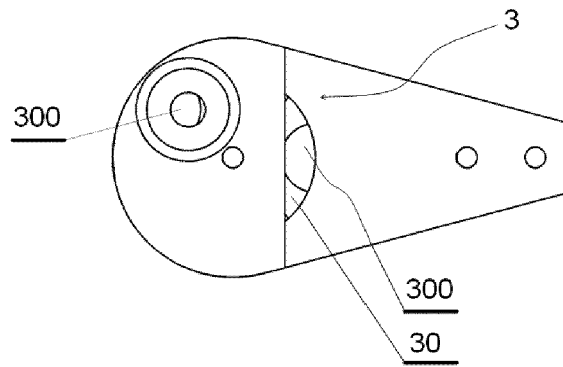


Fig. 2b

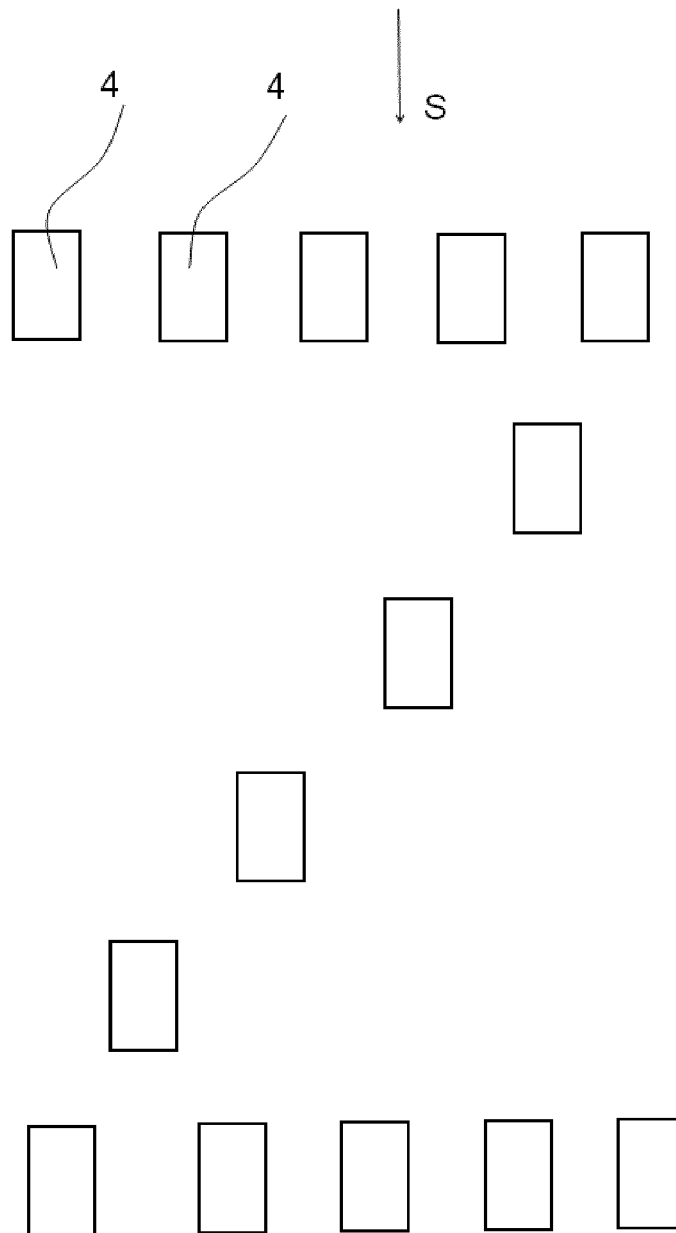


Fig. 3

REFERENCES CITED IN THE DESCRIPTION

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